A New Frontier for Plant Modification

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Should we be Trying to (Bio)Genetically Modify Plants?

Before introducing the reader to the beginning of a new era in lignin modification in particular and plant modification in general, a few comments regarding genetic engineering of plants are perhaps in order. Although the ethical issues are complex and need to be aired elsewhere, it is worth remembering that humans have exploited plants throughout history. Nearly every fruit, vegetable, and grain grown today for human consumption is far from its native cousin. Modern plants have been forced to evolve along a specific path that benefits our needs. The continued enjoyment of eating seedless fruits is not the result of natural selection but rather man's careful selection and crossing based on a sound understanding of the plants' genetics. By their very definition, such plants are doomed to rapid extinction in the natural world. While some have problems with the idea of taking a gene from one plant and putting it into another, they have no problems with grafting a prized apple stem onto a hardy root stock of some completely different variety. And let's not forget that nature, with the aid of cosmic rays and other mechanisms, has already explored many such options herself. There is little doubt that biogenetic engineering will produce a variety of more exploitable plants.

What are the Benefits of Engineering Lignin?

What benefits are to be gained from engineering lignin? In the dairy forage arena, the biggest aim is toward improved forage digestibility. Certainly, the ruminant animal needs an indigestible component, but much of the potentially digestible polysaccharides in the forage cell wall are inaccessible to that ruminant. To put cell wall digestibility gains in perspective, other factors being equal (an assumption), a 10% improvement in forage cell wall digestibility would result in some $350 million in increased U.S.milk and meat production, while producing 2.8 million tons less manure solids, and requiring 2 million tons less supplementary grain.

One of the mechanisms limiting digestibility highlighted by work in the Cell Wall Group is chemical cross-linking of polysaccharides to the indigestible lignin component. What if we could down-regulate cross-linking by genetic engineering? Hans Jung and Weting Ni are exploring such an approach. If post-harvest treatments are considered, altering the lignin or the cross-linking to make polysaccharides more available is also an option.

Modifications to lignin has benefits beyond the forage industries. The chemical pulping industry, producing paper from wood and agricultural feedstocks, would save $billions if the cooking temperature or time in the digester could be reduced. Pulping is the process by which lignin is removed from the desired cellulose component.

Current Approaches to Changing Lignin

There is considerable activity in trying to down-regulate lignification, particularly targeted toward the pulping industry. Since lignin is the polymer that must be removed to make quality white paper, its down-regulation seems attractive. Of course, lignin serves a variety of functional roles in the plant so its manipulation is not without costs in plant vigor. To date, antisense techniques have been used to down-regulate many of the enzymes on the lignin monomer biosynthetic pathway. For only a few of those has the actual amount of lignin been reduced — as will be explained below; depriving the plant of its ability to produce traditional lignin monomers does not necessarily thwart its ability to produce 'lignin.' A case can be made for the opposite approach, that of increasing lignin. Such approaches may provide

additional commodities, higher fuel-value plants, and a potential for a decreased use of pesticides in plants with improved pest resistance.

Recent Findings

We recently made some startling discoveries concerning the lignin in a natural pine mutant that have opened up a new frontier for plant modification (Ralph et al., 1997). That mutant, identified by workers at NC State (MacKay et al., 1997), had a CAD enzyme activity 1% or less of wild type. We isolated a lignin fraction to carefully examine the lignin structure by NMR. The spectra were immediately striking. In addition to the obvious decline in units derived from coniferyl alcohol, the lignin had enhanced aldehyde levels (as predicted), including new aldehyde components that had never been reported in plant lignins, and a predominant component derived from dihydroconiferyl alcohol. Dihydroconiferyl alcohol is not associated with the normal monolignol biosynthetic pathway and was not anticipated in the polymer.

Two aspects of the findings have generated considerable excitement (and controversy) and the Science article has been followed up and popularized (not always accurately!) by several publications (Boudet, 1997; Kling, 1997; Anon., 1997; Ralph, 1997). First, the findings begin to explain prior mysteries regarding the normal levels of "lignins" produced by plants whose genes were so heavily down-regulated; the down-regulated plants simply create their lignins from other available phenolic components. Second, they suggest enormously increased potential for improved utilization of plants through genetic engineering of plant lignins.

Opposing View

There are those who claim that lignification is a highly regulated process incorporating only the three hydroxycinnamyl alcohols into lignin. The evidence for this position is a tenuous extrapolation of fascinating lignan observations (Davin et al., 1997); it faces continually mounting evidence for the contrary view, i.e., that many other components incorporate into lignins in a wide variety of 'normal' plants and certainly in stressed and mutant plants. When you can take two plant materials, isolate a fraction by identical methods, and obtain polymeric fractions that are clearly different, then the plants must be metabolically different. But is this product lignin? Certainly not if you define lignin as a strict polymer of hydroxycinnamyl alcohols (see following paper). But we have sufficiently demonstrated that these "lignins" with substantial amounts of nonconventional components are formed by the plant using radical coupling methods analogous to those that typify lignification. It is reasonable to assume that the plant is utilizing this polymer for the same purposes as it uses lignin. Whether we want to continue to call it lignin or not is just semantics. Plants appear to have considerable plasticity in producing a polymeric material that functions as "lignin."

Implications for Genetic Engineering of Lignin

The ability to produce functional lignins or lignin equivalents from nontraditional monomers, Figure 1, opens up enormous new opportunities for engineering lignins for better utilization of plant materials. Researchers are no longer restricted to the enzymes and components of the traditional lignin biosynthetic pathway. The range of properties that can be engineered is not yet known, but there is reason to assume that engineering lignins to be more extractable, allowing easier pulping or providing opportunities for post-harvest treatments to improve forage digestibility, is among the likely outcomes.

Conclusions

Evidence suggests that current assumptions about the process of lignification are too narrow. A remarkable range of 'nontraditional' phenols, i.e., phenols other than the three hydroxycinnamyl alcohols, are incorporated into the lignins of a variety of plants. Various mutant plants have unanticipated responses to impositions placed on them by down-regulation, by various means, of normal pathway enzymes. Deprived of their ability to produce normal monolignols, these plants utilize other phenolic compounds to produce a modified 'lignin' that appears to provide the basic

mechanical and water transport functions that allow the plants to survive. Consequently, mutants provide a rich, largely untapped, source of insights into biosynthetic pathways and the metabolic plasticity of lignification. Exploring the breadth of the lignification process by studying the structure of lignins from lignin-biosynthetic-pathway-mutants will not only improve our understanding of lignin pathways but will provide new targets for biogenetic engineering to produce plants with exploitable characteristics.

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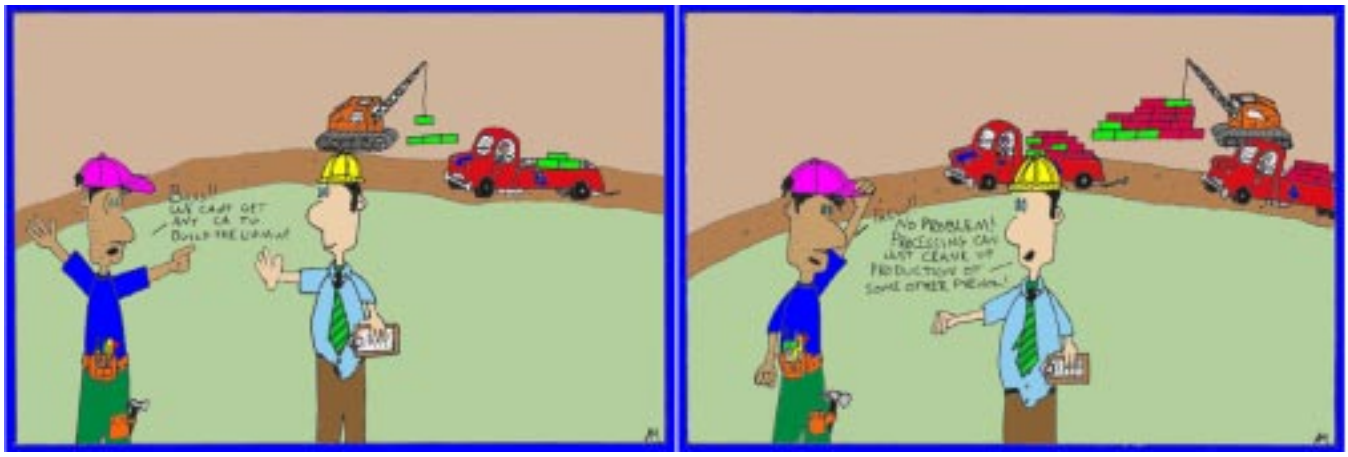

Boss!! We can't get an CA (coniferyl alcohol) to build the lignin!

No problem! Processing can just crank up production of some other phenol! Whew!

Figure 1. This cartoon, drawn by nephew Andy Muenchow, 13, was supposed to be provocative. We do not yet know what other phenols a plant may be able to incorporate into its lignin, but we do know that phenols that are not normally associated with the lignin biosynthetic pathway are incorporated into 'lignins' in a range of normal and genetically modified plants. Certainly, the currently held views of lignification (i.e., that lignins are built from only three hydroxycinnamyl alcohol monolignols) are too narrow. With modifications possible beyond the traditional monomer pathway, there is considerable new potential for biogenetically modifying lignin's components and structure to our advantage.